Reference: *Biol. Bull.* **187:** 398–407. (December, 1994)

Energy Metabolism and Amino Acid Transport During Early Development of Antarctic and Temperate Echinoderms

FRASER M. SHILLING[1] AND DONAL T. MANAHAN[2]

Department of Biological Sciences, University of Southern California, Los Angeles, California 90089-0371

Abstract. The rates of oxygen consumption by embryos of antarctic echinoderms (*Acodontaster hodgsoni*, *Odontaster validus*, *Psilaster charcoti*, and *Sterechinus neumayeri*) were compared to the biomass (ash-free dry organic weight) of the egg of each species. These species could survive for months to years (range: 10 months to 5 years) by relying solely on the reserves present in the egg. However, certain species did not use any of the egg's reserves during early development. Embryonic stages of *O. validus* (a species with planktotrophic larvae) did not decrease in lipid, protein, or total biomass during the first 35 days of development. During the first 42 days of development, embryos of *A. hodgsoni* (a species with lecithotrophic development) used protein as an energy source. For both species lipid composed 40 to 50% of egg biomass, but was not used as an energy reserve. Larvae of *O. validus* have a high-affinity transport system for amino acids dissolved in seawater (K_t = 1.3 μM for alanine). The rate of alanine transport from a low concentration (50 nM) could supply 32% of the larva's metabolic needs. This is a 10-fold higher input to metabolism than was determined (3% at 50 nM) for larvae of a temperate asteroid, *Asterina miniata*. Larvae of antarctic echinoderms live in an environment where the food supply is low for most of the year. Relative to their metabolic rates, antarctic larvae have larger energy stores and planktotrophic larvae have higher nutrient transport capacities when compared to larvae from temperate regions. These physiological differences allow antarctic larvae to survive for long periods without particulate food.

Received 4 June 1993; accepted 26 September 1994.

[1] Current address: Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, L-474, Portland, OR 97201.

[2] Address for correspondence.

Introduction

Many species of marine invertebrates in Antarctica have lecithotrophic (nonfeeding) stages of development (Pearse *et al.*, 1991). For antarctic species that have planktotrophic (particle-feeding) larvae, the strategies for survival in a low-food environment remain unknown. For example, *Odontaster validus* is a very abundant echinoderm (asteroid) in McMurdo Sound, Antarctica, and has a planktotrophic larval stage (Pearse, 1969). The adults spawn in austral winter (June–September: Pearse *et al.*, 1991) and larvae are present for several months in a water column that has very low concentrations of phytoplankton (*ca.* 0.01 μg chlorophyll *a* l^{-1}, Rivkin, 1991). Yet, when cultured *in situ* in the water column, larvae of this species survive for weeks to months (Olson *et al.*, 1987) prior to the phytoplankton bloom of austral summer (December–January, Rivkin, 1991).

The present study was undertaken to determine the physiological bases for the survival of antarctic echinoderm larvae living under low-food conditions. It has been suggested that, in the near-absence of algae, larvae of antarctic echinoderms could be using other sources of food, such as bacteria or organic material dissolved in seawater (Rivkin *et al.*, 1986). In addition, larvae could be endowed with large amounts of energy reserves from the egg. This would permit survival in a nutrient-poor water column if the larvae have lower metabolic rates (*cf.* temperate larval forms) and correspondingly lower rates of utilization of energy reserves. In the present study, egg mass and metabolic rates were measured for four species of antarctic echinoderms to allow for a calculation of life span in the absence of any exogenous food source. Embryos of *Acodontaster hodgsoni* (a species with lecithotrophic development) and *O. validus* were also cultured through em-

bryogenesis to determine actual rates of utilization of biomass (ash-free dry organic weight) and energy reserves (lipid and protein content). To assess the possible role of dissolved organic material as a source of nutrition for larvae, the mass-specific rates of amino acid transport from seawater were compared with the mass-specific metabolic rates of larvae. These physiological processes (amino acid transport and metabolic rate) were measured for larvae of *O. validus* and compared to the measurements made for similar larvae of a temperate asteroid (*Asterina miniata*).

Materials and Methods

Culturing of embryos and larvae

Adult echinoderms were collected using scuba from several locations in McMurdo Sound, Antarctica, during austral spring (asteroids: *Acodontaster hodgsoni, Odontaster validus, Perknaster fuscus, Psilaster charcoti;* and the echinoid, *Sterechinus neumayeri*). Prior to spawning, adults were maintained in ambient seawater (on sea tables) for up to 2 weeks in the aquarium at McMurdo Station. Gametes were obtained from adult asteroids by intracoelomic injection of 1 m*M* 1-methyladenine. Gametes from sea urchins were obtained by intracoelomic injection of 0.5 *M* KCl. Fertilized eggs of the antarctic species were suspended in 200-l vessels (Nalgene) of filtered seawater (0.2 μm pore-size). Eggs (and later the embryos and larvae) were kept in suspension by mixing the cultures with vertically moving Plexiglas paddles driven by electric motors set at slow speeds (5–10 rpm). Low concentrations of individuals were maintained in the culture vessels (*e.g., O. validus* was cultured at 2–3 individuals ml^{-1} and *A. hodgsoni* at 0.1 individuals ml^{-1}). The temperature of the cultures was maintained by immersing the 200-l culture vessels in tanks of flowing ambient seawater (mean temperature of −1.2°C). The culture seawater was replaced every 4 to 5 days with newly filtered seawater, at which time the vessels were cleaned by acid-washing. For *O. validus,* one batch of eggs (a total of 1.5 million eggs obtained from several females) was divided equally among three replicate 200-l culture vessels to ensure sufficient numbers of individuals in each vessel for measurements of biomass, biochemical composition, and metabolic rates. Embryos and larvae of the temperate species (*Asterina miniata*) were cultured in California at 14°C. Gametes of this species were obtained from ripe adults purchased from Marinus Inc., Long Beach, California.

Biochemical composition of antarctic eggs (4 species) and changes in biomass during early development of A. hodgsoni *and* O. validus

Prior to sampling, all of the embryos or larvae from a single culture were slowly siphoned from the culture vessel and concentrated onto a nylon screen (80 μm, mesh size) that was partially immersed in ambient seawater. At each change of seawater, three to six samples were taken for the measurement of biomass (ash-free dry organic weight) and an additional three samples were taken for biochemical composition (lipid and protein content). Salts were removed from the samples using serial washes of an ammonium formate solution (methods described previously by Jaeckle and Manahan, 1989). Protein content was measured with the Bradford assay (1976). Trichloroacetic acid-insoluble protein was dissolved by heating (60°C) the protein pellet in 1 *M* NaOH (aq.). This method results in a close (7%) approximation of the absolute amount of protein, as determined by measuring with high-performance liquid chromatography the total amino acid content of acid-hydrolyzed protein homogenates of larvae (Manahan *et al.,* unpub. obs.). Lipid was measured according to Mann and Gallager (1985). Carbohydrate content was not measured in our studies because carbohydrate is a low percentage (2–4%) of the total organic material in eggs of antarctic echinoderms (McClintock and Pearse, 1986), as has been reported for eggs and larvae of other species of marine invertebrate (Holland, 1978).

Metabolic rates of embryos and larvae

Rates of oxygen consumption were measured using Strathkelvin (Glasgow, U.K.) polarographic oxygen sensors (Model 1302) that were connected to oxygen meters (Model 781). The output (as volts) from each meter was recorded by an IBM computer using a software package for data acquisition and analysis (Datacan, Sable Systems Inc., Los Angeles, California). Two or three respiration chambers (Strathkelvin, Model RC 200) were used simultaneously and two to six independent measurements of respiration were made using different groups of embryos or larvae at the same stage of development.

Rates of oxygen consumption were measured for the antarctic species at either −1.4°C or −2.0°C (see Results for corresponding temperature and experiment). Oxygen consumption was measured on the same day that samples were taken for biomass and biochemical composition, or when rates of alanine transport were determined. Prior to any series of measurements on a given day, the respiration chamber and the membrane of the oxygen sensor were washed with 75% ethanol to minimize bacterial activity, and then rinsed four times with filtered seawater. At temperatures of −1.4 or −2.0°C, the oxygen sensors took an average of 2–3 h to stabilize when filtered seawater (with no animals added) was present in the respiration chamber. To obtain reliable data for the antarctic species, all respiration rates were determined only when at least 2 h had elapsed after the animals were placed into the respiration chamber. Rates of oxygen consumption for

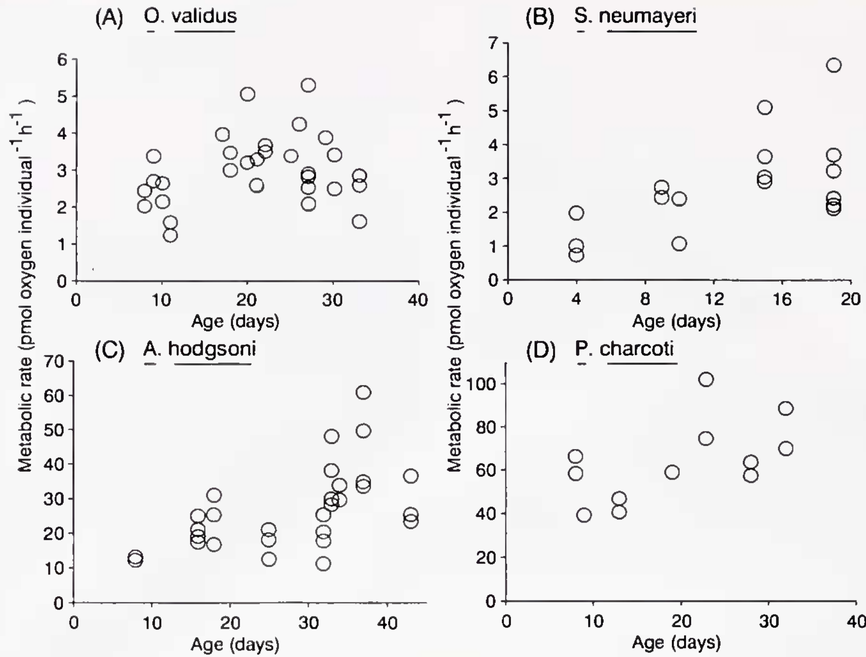

Figure 1. Rates of oxygen consumption by the developing stages of antarctic echinoderms. (A) *Odontaster validus;* (B) *Sterechinus neumayeri;* (C) *Acodontaster hodgsoni;* and (D) *Psilaster charcoti.* Each data point represents a single determination of metabolic rate over a period of 1–2 h.

larvae of the temperate species (*A. miniata*) were determined at 12°C using the same respiration apparatus as was used for the antarctic species. At these higher temperatures the oxygen sensors needed only 30 min to stabilize, allowing measurements to be made over the subsequent 30–60 min.

The volume of each of the respiration chambers (Model RC 200) was calibrated with an Eppendorf pipettor and set at 50 μl for all the antarctic species and 100 μl for the temperate species. At the end of each measurement, the animals were removed from the respiration chamber and counted. The number of animals used in the chamber depended upon the species. For the antarctic echinoderms, the number of individuals used ranged from 5 to 20 for the larger species (*A. hodgsoni* and *P. charcoti*) and from 33 to 221 for the smaller species (*O. validus* and *S. neumayeri*). For *A. miniata,* 29 to 93 larvae were used per chamber. The rate of oxygen depletion per individual was calculated by determining the rate of respiration of all the embryos or larvae in the chamber and dividing that rate by the number of individuals present. Rates of oxygen consumption with no animals present were determined as controls for each set of measurements and were subtracted from the rates where animals were present. For each set of measurements two control measurements were done, one immediately before and one after those measurements made with animals present. For all measurements (n = 97), the changes in oxygen tension in the absence of animals (controls) ranged from 5 to 25% of those rates with animals present (experimental). Data were used only if they had a signal-to-noise ratio of at least 3-to-1; *i.e.,* the rate with larvae was at least 3-fold greater than the rate without larvae (controls).

Calibration and accuracy of polarographic oxygen sensors at low temperatures

The voltage output from each oxygen sensor was calibrated to the concentration of oxygen in seawater (at the experimental temperatures) by immersing the sensor in a BOD (biological oxygen demand) bottle containing 300 ml of seawater. The voltage readings from the oxygen sensors were recorded and the amount of oxygen present in the BOD bottle was measured by Winkler's titration (Parsons *et al.,* 1984).

The measured metabolic rates of the antarctic embryos and larvae were low (see Fig. 1) and often near the limit of detection of the polarographic oxygen sensors (signal-to-noise ratio of 3:1). The following procedure was used to check that the low rate of oxygen depletion that was observed was not an artifact of any abnormal functioning of the sensors at the cold temperature of antarctic seawater. The biological depletion of oxygen at −2.0°C was mimicked by bubbling helium into seawater. Up to 50% of the oxygen was removed from seawater that had been placed in a series of seven BOD bottles. The voltage read-

ing was recorded from the oxygen sensor placed in each BOD bottle containing reduced oxygen and the actual amount of oxygen present in each seawater sample was then measured chemically with Winkler's titration. The decrease in the oxygen content of seawater measured with the sensors was not significantly different from the decrease as measured independently by Winkler's titration (least-squares linear regression analysis, variance ratio of slope = 0.35^{ns}, $n = 7$).

Measurement of alanine transport rates by antarctic and temperate larvae

Time course experiments were done at a range of different alanine concentrations to determine the kinetics of amino acid transport by larvae of *O. validus* (at −2.0°C) and *A. miniata* (at 12.0°C) (methods previously described by Manahan *et al.*, 1989). Radioactive alanine (^{3}H-alanine, 84 Ci mmol^{-1}, New England Nuclear) was added (2 or 5 μCi) to 10 ml of seawater in the presence of a range of concentrations (0.1–100 μM) of nonradioactive alanine (Sigma Chemical Co.). Aliquots (500 μl) containing larvae (*O. validus:* 222 ml^{-1}; *A. miniata:* 322 ml^{-1}) were taken from the 10-ml vial every 2 min for 20 min. The transport rate of alanine at a given concentration was calculated (as pmol alanine larva^{-1} h^{-1}) using (1) the measured rate of accumulation of radioactivity (as disintegrations per minute after quench correction), (2) the specific activity of the radiolabel, and (3) the number of larvae per sample. The rate of alanine transport per larva as a function of increasing alanine concentration was plotted to determine the affinity (K_t) and maximum capacity (J_{max}) of the amino acid transporter in antarctic and temperate larvae.

Results

Metabolic rates during early development

During early development of *O. validus*, from the blastula (8 days) to late gastrula stage (27 days), there was an increase in metabolic rate (Fig. 1A) measured at −1.4°C. The significance of this increase was determined using analysis of variance for the data during that 19-day period (variance ratio, VR = 6.80*, $F_{0.05[1,23]} = 4.28$). The data for early bipinnaria (after 27 days) were not used in this analysis of early development because the increase in respiration rate did not continue once the larval stage was reached. For *S. neumayeri* (Fig. 1B), there was an increase in metabolic rate (VR = 8.28*, $F_{0.05[1,15]} = 4.54$) from the unhatched blastula (4 days) to the gastrula stage (19 days). The larval stage of *A. hodgsoni* (a bilobed bipinnaria, Bosch and Pearse, 1990) had developed by 36 days. From the early embryo (8 days) to the larval stage (36 days) there was an increase (VR = 14.41***, $F_{0.05[1,24]} = 4.26$) in metabolic rate (Fig. 1C). However for *P. charcoti*, a species with lecithotrophic development, no significant change was measured in metabolic rate during the period of development studied (to 32 days) (Fig. 1D, VR = 4.56^{ns}, $F_{0.05[1,10]} = 4.96$). The lack of statistical significance may be an effect of a smaller sample size for this species.

The metabolic rates of the bipinnaria larvae used for the comparison of metabolic rates and amino acid transport rates were 27.5 ± 1.51 pmol O_2 larva^{-1} h^{-1} ($n = 6$) for 5-day-old larvae of *A. miniata* (measured at 12°C) and 2.22 ± 0.69 pmol larva^{-1} h^{-1} (n = 4) for 42-day-old larvae of *O. validus* (−2°C). These different ages of larvae for the two species represent the same stage of development (early bipinnaria) because of the slower development rate of *O. validus* relative to *A. miniata*. Errors given here and throughout this paper (unless otherwise stated) are one standard error of the mean (±1 SEM).

Scaling of metabolic rates with biomass during early development of antarctic echinoderms

The scaling of metabolic rate to ash-free dry organic weight (biomass) for embryos of *S. neumayeri, O. validus, A. hodgsoni*, and *P. charcoti* is shown in Figure 2A. When the log of metabolic rate (mean of all values for each species from Fig. 1) was plotted against the log of biomass (mean for each species), a slope of 0.66 ± 0.213 (95% confidence limit) was calculated. These metabolic data can be described by the power function:

$$\text{Metabolic rate} = 3.14\ M^{0.66}$$

where metabolic rate is given as pmol O_2 individual^{-1} h^{-1} and mass (M) has units of μg ash-free dry organic weight.

Volume to biomass relationship, and lipid and protein content of eggs of antarctic echinoderms

The slope of the line describing the relationship of egg biomass to egg volume is 0.917 (Fig. 2B). The egg diameters for the five species used in this comparison were obtained from the following reports: *S. neumayeri* from Bosch *et al.* (1987), and *A. hodgsoni, O. validus, P. fuscus*, and *P. charcoti* from Bosch and Pearse (1990). Values for biomass and egg diameters used to construct Figure 2B are given in Table IA for four of the five species (egg volumes based on a sphere). The data point for the eggs of *P. fuscus* is based on the following values: biomass = 87.9 ± 1.07 μg, diameter = 1200 μm, volume = 904.7 × 10^{-3} mm^3.

The percent of the ash-free dry organic weight of eggs that was composed of lipid and protein was calculated for four asteroid species (Fig. 2C). Eggs of *O. validus* had equal proportions of lipid and protein, whereas eggs of the three other species (all have lecithotrophic development) had a greater proportion of biomass as lipid. The

percent recovery of dry organic weight as lipid and protein ranged from 65% (*P. charcoti*) to 89% (*P. fuscus*)—*i.e.*, 11 to 35% of total dry organic weight was not accounted for by the lipid and protein content of the eggs.

Changes in biomass, lipid, and protein content during early development of O. validus *and* A. hodgsoni

Embryos of *O. validus* in the three replicate cultures had similar changes in biomass, lipid, and protein content from the egg (0 days) to early bipinnaria larva (35 days) (Fig. 3A). As stated earlier, one batch of eggs obtained from several females was used to start all three of the 200-l cultures. Biomass increased significantly (ANOVA, VR = 83.13***, $F_{0.05[1,21]} = 4.32$) from the egg (0 days) to the early gastrula stage (pooled data for 9, 10, and 12 day-old) and thereafter remained fairly constant (*i.e.*, no significant difference between gastrula and early larval stage at d 35, VR = 2.24^{ns}, $F_{0.05[1,32]} = 4.12$). The equation for the fitted curve through all the biomass data points is given in the figure legend. Linear regression analyses were used to determine the rates of lipid and protein utilization during early development of *O. validus* (regression lines excluded for graphical simplicity, Fig. 3A). Lipid and protein content did not change significantly (determined by ANOVA) from the egg to early larva ($VR_{protein} = 0.22^{ns}$, $VR_{lipid} = 0.09^{ns}$, $n = 22$).

For the first 42 days of development of *A. hodgsoni*, lipid content did not change (Fig. 3B, VR = 0.01^{ns}, $n = 25$). Both protein content and biomass did, however, decrease during this period. Protein decreased at a rate of 25.7 ± 8.8 ng individual^{-1} day^{-1} (VR = 8.60**, $F_{0.05[1,21]} = 4.32$). Biomass decreased at a rate of 52.4 ± 14.3 ng individual^{-1} d^{-1} (VR = 13.49**, $F_{0.05[1,23]} = 4.28$).

Transport of alanine from seawater by larvae of A. miniata *and* O. validus

The larvae of *A. miniata* had a maximal transport capacity (J_{max}) for alanine of 17.15 pmol μg^{-1} h^{-1} (at 12°C), whereas the J_{max} for alanine by larvae of *O. validus* was 6.51 pmol μg^{-1} h^{-1} (at −2°C) (Fig. 4A). The affinities (values of K_t) of the alanine transport systems in larvae of both species were similar (see slopes, Fig. 4B), at 1.9 μM for *A. miniata* and 1.3 μM for *O. validus*.

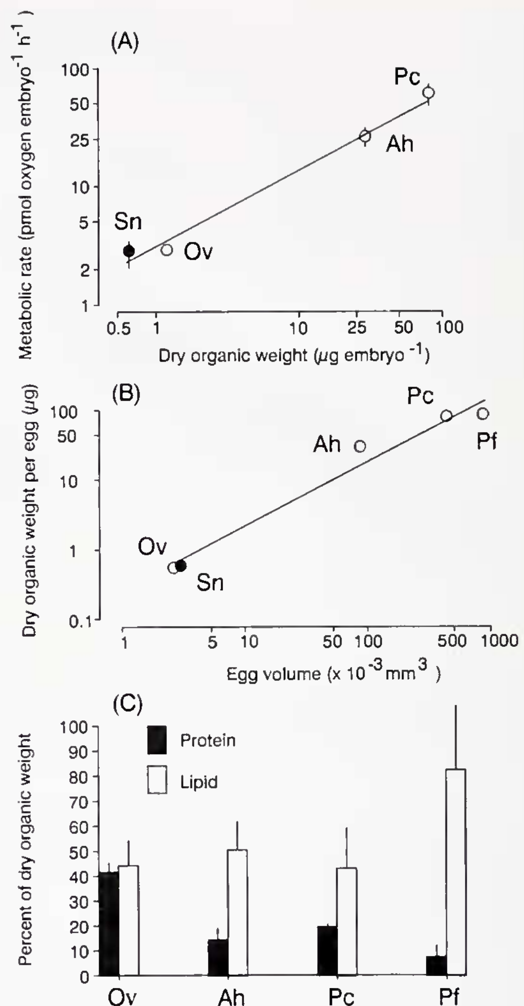

Figure 2. Metabolic scaling, relationship of egg volume to biomass (ash-free dry organic weight), and lipid and protein content of eggs of antarctic echinoderms. Ah, *Acodontaster hodgsoni;* Ov, *Odontaster validus;* Pc, *Psilaster charcoti;* Pf, *Perknaster fuscus;* Sn, *Sterechinus neumayeri* (echinoid, closed circles). (A) Change in metabolic rate as a function of embryos' biomass, error bars represent 95% confidence limits of the mean, where no error bars are shown errors fell within graphical representation of data point. (B) Relationship of egg biomass to egg volume. (C) Lipid and protein content of eggs as percent of biomass with 95% confidence limits.

Discussion

Lifespan of embryos and larvae

Antarctic echinoderm larvae have to survive periods of many months in a nutrient-poor water column (Olson *et al.*, 1987; Pearse *et al.*, 1991; Rivkin, 1991). Survival under such conditions should be difficult, especially for those species with larval forms that require food to complete development (*e.g.*, *O. validus* and *S. neumayeri*). An estimate of larval life span under starvation conditions can be obtained by calculating how long the maternal endowment of energy reserves initially present in the egg could supply the metabolic demand (rates of respiration) of embryos and larvae. Starvation conditions are defined here as the absence of any dietary inputs, either in particulate form or as dissolved organic material. The energy available as biomass in the eggs of species with planktotrophic and lecithotrophic modes of development was compared to the metabolic rates of those same species during early development (Table 1). The time it would take to deplete

Table I

Calculation of the duration of larval life span: (A) for the antarctic echinoderms Acodontaster hodgsoni, Odontaster validus, Psilaster charcoti, *and* Sterechinus neumayeri. *(B) For temperate echinoderms* Asterina miniata *and* Strongylocentrotus purpuratus

(A) Antarctic species:				
Species	*O. validus*	*S. neumayeri*	*A. hodgsoni*	*P. charcoti*
Egg size[1] (μm)	170	179	550	950
Egg biomass[2] (μg ± SEM)	0.63 ± 0.018	0.69 ± 0.032	30.1 ± 0.06	81.3 ± 1.91
Metabolic rate[3] (pmol O_2 ind.$^{-1}$ h^{-1})	2.94 ± 0.18	2.90 ± 0.33	26.1 ± 2.26	63.9 ± 5.09
Biomass depletion[4] (ng ind.$^{-1}$ day^{-1})	1.08	1.06	9.55	23.4
Time to deplete 50% of egg biomass (months)	9.8	10.8	52.5	58.0

(B) Temperate species:		
Species	*A. miniata*	*S. purpuratus*
Egg size[1] (μm)	180	70
Egg biomass[2] (μg ± SEM)	0.70 ± 0.01	0.07
Metabolic rate (pmol O_2 ind.$^{-1}$ h^{-1})	27.5 ± 1.51	12.7
Biomass depletion[4] (ng ind.$^{-1}$ day^{-1})	10.1	4.65
Time to deplete 50% of egg biomass (months)	1.16	0.25

[1] Egg diameters from Bosch *et al.*, 1987; Bosch and Pearse, 1990; Strathmann, 1987.
[2] Biomass measured as ash-free dry organic weight, data from this study or from Shilling and Manahan, 1990.
[3] Metabolic rate, data from this study (mean ± SEM) or from Shilling and Manahan (*op. cit.*).
[4] Biomass depletion, ng of dry organic weight (individual)$^{-1}$ (day)$^{-1}$. Each rate of aerobically catabolized biomass was calculated using the mean of the oxyenthalpic equivalents of lipid and protein [484.0 kJ (mol O_2)$^{-1}$] and the specific enthalpy of combustion of lipid and protein (31.75 kJ g^{-1}) (values from Gnaiger, 1983).

50% of the eggs' biomass was calculated. For the antarctic species studied that had the smallest egg biomass (0.63 μg, *O. validus*), 50% of the egg's biomass could provide sufficient energy to meet metabolic demand (2.94 pmol O_2 individual^{-1} h^{-1}) for 9.8 months (Table IA). For *P. charcoti*, an antarctic species that had the largest egg biomass (81.3 μg) in this comparison, a similar calculation revealed that the lecithotrophic larvae of this species could survive for a remarkably long time under starvation conditions—58 months (4.8 years).

The long survival times of antarctic larval forms are in marked contrast to those of temperate species of echinoderm. Larvae of *A. miniata*, starting from a similar egg size to *O. validus*, have an 8.4-fold shorter potential life span (Table IB). Larvae of the sea urchin *Strongylocentrotus purpuratus* would deplete 50% of the egg's biomass in only 8 days. In summary, the potential life spans of larvae of the antarctic species are in the range of months to years, while those of the temperate species are much shorter at days to weeks. This comparison of life spans of antarctic and temperate echinoderm larvae suggests that antarctic larval forms living in an environment that is episodic with respect to food availability have a "wait-and-see" strategy of being able to withstand starvation conditions for periods of months to years. This strategy is not available to temperate larval forms and tropical larval forms when developing at temperatures characteristic of their respective habitats, at least for echinoderms. The tropical species do not have correspondingly larger egg sizes (Emlet *et al.*, 1987) when compared to eggs of antarctic echinoderms (Fig. 2B; see also McClintock and Pearse, 1986; Bosch *et al.*, 1987; Bosch and Pearse, 1990) or temperate species (McEdward and Chia, 1991).

Transport of dissolved amino acids and relationship of transport capacity to metabolic rate

The above calculations of life span show one possible physiological mechanism for survival of antarctic echinoderm larvae under low-food conditions. A comparison of nutrient uptake rates and metabolic rates of antarctic and temperate larval forms revealed a second possibility: antarctic larvae have a high nutrient uptake capacity relative to metabolic rate when compared to a temperate larval form (Table II). The bipinnaria larvae of the antarctic and temperate species of asteroid chosen for this physiological comparison develop from eggs of similar sizes (*O. validus*, 0.63 ± 0.018 μg; *A. miniata*, 0.697 ± 0.007 μg). At the appropriate temperature for each species (−2.0 and 12.0°C) the larvae of these two species had a 20.6-fold difference in mass-specific metabolic rates (2.22 *cf.* 45.75 pmol O_2 μg^{-1} h^{-1}). However, the mass-specific capacity for alanine transport (J_{max}) was only 2.6-fold higher for the temperate species (6.51

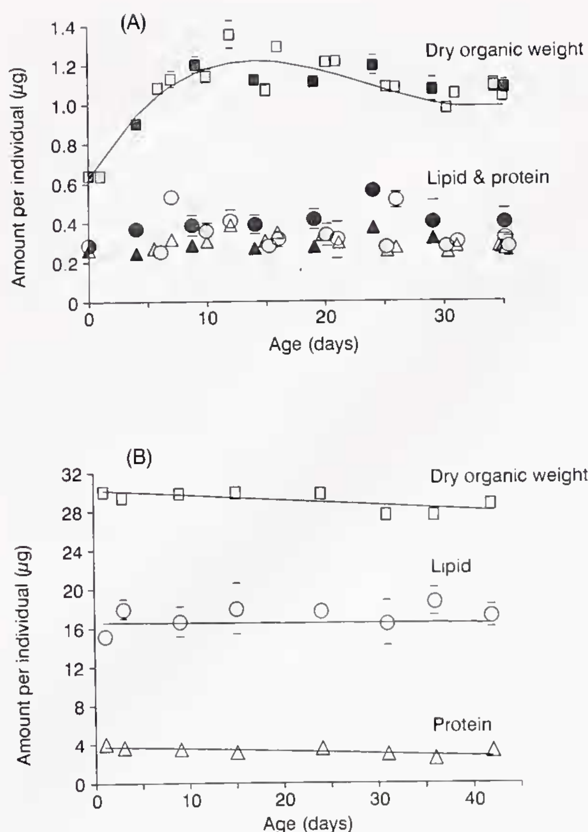

Figure 3. Changes in dry organic weight, lipid, and protein content of embryos of (A) *Odontaster validus* and (B) *Acodontaster hodgsoni*. Squares, ash-free dry organic weight (biomass); circles, lipid; triangles, protein. For all graphs error bars represent one standard error of mean (SEM), where bars are not shown the error fell within graphical representation of data point. (A) Open, stippled, and solid symbols represent three different cultures reared from the same batch of eggs. Curve was fitted through all biomass data points (n = 122, including replicates) using the equation: $Y = (0.6236) + (0.0976X) + (-0.00495X^2) + (0.00007X^3)$, where Y is ash-free dry organic weight (µg) and X is time (days). (B) Lines represent slopes calculated from least-square regression analyses.

cf. 17.15 pmol alanine $\mu g^{-1} h^{-1}$). Both species had a high affinity for amino acid transport (K_t = 1.3 or 1.9 μM). Alanine was chosen for these studies because it is transported by developing marine invertebrates at a rate that is close to the average when a mixture of amino acids is present (Manahan, 1989).

The consequence for the antarctic species of having a higher-capacity transport system relative to metabolic rate, compared to the temperate species, is that the former could gain substantial metabolic benefit from the transport of amino acids present at low concentrations. The amino acid concentrations in waters of McMurdo Sound, Antarctica, were measured with high-performance liquid chromatography from austral winter (August) to austral summer (January) (Welborn and Manahan, 1991). Total amino acid concentrations in the water column prior to the phytoplankton bloom in December were low, ranging from below the limit of detection of the analysis to *ca.* 100 nM. If larvae of *O. validus* were in an environment with 50 nM alanine, the rate of alanine transport could supply 32% of the larva's metabolic rate (Table II). In contrast, larvae of the temperate species could supply only 3% of their metabolic rate at 50 nM alanine (*A. miniata*, Table II). A contribution of ⅓ of metabolic rate for a marine invertebrate is a very high metabolic input from transport of dissolved amino acids alone at a concentration of only 50 nM (see reviews by Stephens, 1988; Wright, 1988; Wright and Manahan, 1989; Manahan, 1990). For the temperate species to supply ⅓ of metabolic rate, larvae of *A. miniata* would require a 15.8-fold higher substrate concentration of 788 nM. At the maximal transport capacity (J_{max}) of alanine, larvae of *A. miniata* accounted for *ca.* 100% of metabolic needs (Table II). Similar matching of nutrient maximum transport rates with metabolic needs has been found during larval development of the mollusc *Crassostrea gigas* (Manahan *et al.*, 1989) and in vertebrate intestines (Ferraris and Diamond, 1989; Buddington and Diamond, 1989). In contrast, the antarctic larvae (*O. validus*) have a much higher ratio of nutrient transport to metabolic rate (Table II), with the moles

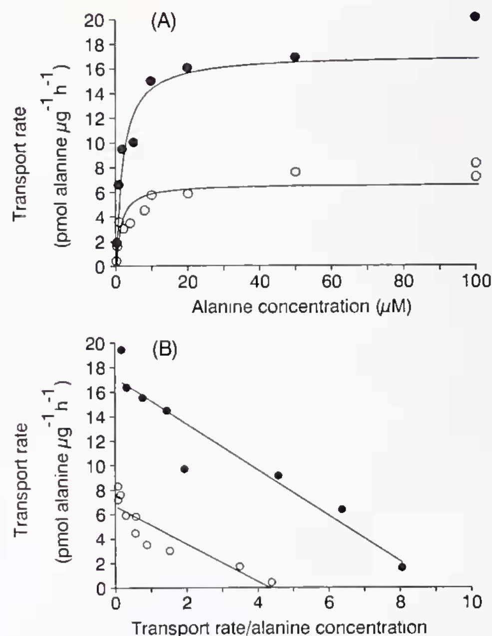

Figure 4. Kinetics of alanine transport by bipinnaria-stage larvae of *Asterina miniata* (12°C, closed circles) and *Odontaster validus* (−2°C, open circles). Values for biomass of larvae given in Table II. Each point represents the transport rate obtained from a separate time course experiment based on 10 samples. The Michaelis-Menten equation was used to plot the saturation curves in (A) using values for K_t and J_{max} for each species (see text) obtained from linear regression analysis of Eadie-Hofstee plots (B).

Table II

Mass-specific metabolic rates and mass-specific rates of alanine transport for bipinnaria larvae of Odontaster validus *(42-day-old) and* Asterina miniata *(5-day-old)*

	O. validus (−2°C)	*A. miniata* (12°C)
Metabolic rate[1]		
(pmol oxygen $\mu g^{-1} h^{-1}$)	2.22	45.75
(pmol Ala-equivalents $\mu g^{-1} h^{-1}$)	0.74	15.25
Transport rate[2] (pmol alanine $\mu g^{-1} h^{-1}$)		
maximum transport rate (J_{max})	6.51	17.15
transport rate at 50 n*M* alanine	0.24	0.45
Percent of metabolic rate as alanine equivalents supplied by transport from 50 n*M*	32.4%	3.0%
Ratio of maximum transport rate (J_{max}) of alanine to metabolic rate as alanine equivalents	8.8	1.1

[1] Mass (ash-free dry organic weight) of *O. validus* was 1.0 μg; *A. miniata* was 0.6 μg. Alanine equivalents of oxygen consumption based on stoichiometry that complete combustion of 1 mole alanine requires 3 moles O_2. Errors for metabolic rates given in "Results."

[2] Calculated with Michaelis-Menten equation using values of K_t and J_{max} for alanine transport by each species (see Fig. 4B for graphical estimates of errors).

of alanine transported at J_{max} accounting for 8.8-times metabolic needs.

Comparisons with previous research

Except for one species *(P. fuscus),* the linear relationship of ash-free dry organic weight of eggs as a function of egg volume (Fig. 2B) is consistent with previously published values of egg diameters of antarctic echinoderms (Bosch *et al.,* 1987; Bosch and Pearse, 1990). McClintock and Pearse (1986) report a dry weight of 2640 μg (5.9% ash) for an egg of *P. fuscus* (1200 μm diameter), compared with our measurement of egg biomass at 87.9 μg dry organic weight. McClintock and Pearse (1986) used a different technique to obtain eggs than the one we used. In their study, full-grown oocytes were separated from the ovary, dried, and weighed. Freely spawned oocytes were used in our study. Perhaps this difference might have resulted in what we suggest was an overestimate by McClintock and Pearse (1986) of the egg weight for *P. fuscus* relative to eggs from other species of antarctic echinoderms (see Fig. 2B, value of *P. fuscus* at 87.9 μg, *cf.* that data point if weight was 2640 μg even allowing for a 5.9% ash weight).

Olson *et al.* (1987) measured the metabolic rates of bipinnaria-stage larvae of *O. validus* and reported values of 1–2 nl O_2 larva^{-1} h^{-1} over a temperature range of −1.0 to −1.86°C. This metabolic rate is equivalent to 67 pmol O_2 larva^{-1} h^{-1} (equal to 1.5 nl O_2 at STP), which is 2.4-fold greater (per larva) than the metabolic rate of the temperate species *A. miniata* measured at 12°C (Table IB: 27.5 ± 1.51 pmol O_2 larva^{-1} h^{-1}). The data of Olson *et al.* (1987) would lead to the suggestion that the antarctic larvae are "cold-adapted" (*cf.* studies with antarctic fish: Torres and Somero, 1988; Crockett and Sidell, 1990). However, our data do not support that conclusion. On a mass-specific basis, the metabolic rate value of Olson *et al.* is 30-fold greater (based on a 1-μg larva) than the value we measured for bipinnaria of *O. validus* (Table II). Our measurement of metabolic rate for this species is consistent with the measurements for the other three antarctic species (Fig. 2A). Together the metabolic rates for the antarctic species give a metabolic power equation (metabolic rate = $3.14\ M^{0.66}$) consistent with the ⅔ mass exponent as argued by Heusner (1991) to be the relationship between mass and metabolic rate, although a slope of 0.75 (Schmidt-Nielsen, 1984) would fall within the 95% confidence limits of our measurement (0.66 ± 0.213). A major difference in methodology between our study and that of Olson *et al.* is that in the present study the polarographic oxygen sensors (POS) were allowed to equilibrate for 2–3 h in the respiration chamber (with larvae present) prior to taking any measurements, as opposed to 30 min in the study by Olson *et al.* (1987). We found that a 2–3 hour equilibration time was necessary for the rate of oxygen consumption by the POS to be less than the low rates of oxygen consumption of the antarctic embryos and larvae. This may be the cause of the discrepancy between our values and the higher values of Olson *et al.* (1987).

Changes in organic weight and biochemical composition during early development

Previous studies have shown statistically significant increases in dry organic weight during embryogenesis of echinoderms and molluscs (Jaeckle and Manahan, 1989; Shilling and Manahan, 1990; Jaeckle and Manahan, 1992; Shilling and Bosch, 1994). Identical techniques (described in Jaeckle and Manahan, 1989) were used in the present study to measure the surprisingly large increase in organic weight for stages of *O. validus* lacking a digestive system (day 0 to 10, Fig. 3A). There was no corresponding in-

crease in lipid or protein content of *O. validus* during the period of biomass increase. The biomass increase of *O. validus* is probably not due to a gain in carbohydrate, as marine invertebrate eggs and embryos do not contain much of this energy reserve (Holland, 1978; McClintock and Pearse, 1986; Jaeckle and Manahan, 1989; Shilling and Manahan, 1990). An alternative explanation for the observed increase in biomass of *O. validus* is that the increase is an artifact of the measurement techniques used and that the values for biomass are too small for stages less than 5 days old (Fig. 3A). The observed differences between stages might be due to differences in drying procedures. This possibility can be eliminated, however, because all samples from the three different cultures were kept frozen until they were all analyzed (dried and ashed) at the same time. The possibility of growth during early development of antarctic echinoderms deserves consideration and further testing because at this time we can offer no physiological mechanism that might explain the observed large biomass increase of stages of *O. validus* lacking a digestive system. Pending further studies, we stress the simpler interpretation of these data: *none* of the metabolic needs during the early development of *O. validus* can be explained through the catabolism of biomass initially present in the egg.

The possibility of growth for stages lacking a digestive system may be specific to only certain species of antarctic echinoderms. A species that has a lecithotrophic mode of development *(A. hodgsoni)* had no increase in biomass during the first 42 days of development (Fig. 3B). It is noteworthy that lipid content did not decrease during this time, a finding consistent with the data showing no decrease in lipid content during 35 days of development of *O. validus* (Fig. 3A). Although lipid is a major reserve in eggs and larvae of marine invertebrates (Holland, 1978), it is not used during the early development of these antarctic species even though the lipid content is high in eggs of species with lecithotrophic development (McClintock and Pearse, 1986; Fig. 2C of this study). For these two species of antarctic echinoderm, we find no evidence for the widely held view that lipid is the major energy reserve used during development.

In conclusion, developing antarctic echinoderms can survive for months to years by relying solely on the energy reserves present in the egg. In the episodic food-environment of the southern ocean, the larvae could survive long periods of starvation.

Acknowledgments

We are most grateful to Dr. Isidro Bosch for his assistance in collecting the antarctic echinoderms in McMurdo Sound, and for his assistance with larval culturing. We thank Dr. Richard Rivkin for his advice and help during the "Winfly" period on the ice. Our thanks also go to Dr. John Pearse for discussions about conducting this research. Antarctic Support Associates, The U.S. Navy (VXE-6), and The National Science Foundation provided logistic and scientific support in Antarctica. This research was supported by NSF grant 88-20130. One of us (DTM) would like to dedicate this paper to his deceased father, the late Mr. C. A. Manahan.

Literature Cited

Bosch, I., and J. S. Pearse. 1990. Developmental types of shallow-water asteroids of McMurdo Sound, Antarctica. *Mar. Biol.* **104:** 41–46.

Bosch, I., K. A. Beauchamp, M. E. Steele, and J. S. Pearse. 1987. Development, metamorphosis, and seasonal abundance of embryos and larvae of the antarctic sea urchin *Sterechinus neumayeri*. *Biol. Bull.* **173:** 126–135.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.

Buddington, R. K., and J. M. Diamond. 1989. Ontogenetic development of intestinal nutrient transporters. *Ann. Rev. Physiol.* **51:** 601–619.

Crockett, E. L., and B. D. Sidell. 1990. Some pathways of energy metabolism are cold adapted in antarctic fishes. *Physiol. Zool.* **63:** 472–488.

Emlet, R. E., L. R. McEdward, and R. R. Strathmann. 1987. Echinoderm larval ecology viewed from the egg. Pp. 55–136 in *Echinoderm Studies*, Vol. 2, M. Jangoux and J. M. Lawrence, eds. A. A. Balkema, Rotterdam.

Ferraris, R. P., and J. M. Diamond. 1989. Specific regulation of intestinal nutrient transporters by their dietary substrates. *Ann. Rev. Physiol.* **51:** 125–141.

Gnaiger, E. 1983. Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. Pp. 337–345 in *Polarographic Oxygen Sensors: Aquatic and Physiological Applications*, E. Gnaiger and H. Forstner, eds. Springer-Verlag, New York.

Heusner, A. A. 1991. Size and power in mammals. *J. Exp. Biol.* **160:** 25–54.

Holland, D. L. 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. Pp. 85–123 in *Biochemical and Biophysical Perspectives in Marine Biology*, Vol. 4, D. C. Malins and J. R. Sargent, eds. Academic Press, London.

Jaeckle, W. B., and D. T. Manahan. 1989. Growth and energy imbalance during the development of a lecithotrophic molluscan larva *(Haliotis rufescens)*. *Biol. Bull.* **177:** 237–246.

Jaeckle, W. B., and D. T. Manahan. 1992. Experimental manipulations of the organic chemistry of seawater: implications for studies of energy budgets in marine invertebrate larvae. *J. Exp. Mar. Biol. Ecol.* **156:** 273–284.

Manahan, D. T. 1989. Amino acid fluxes to and from seawater in axenic veliger larvae of a bivalve *(Crassostrea gigas)*. *Mar. Ecol. Prog. Ser.* **53:** 247–255.

Manahan, D. T., S. Nourizadeh, and W. B. Jaeckle. 1989. Ontogenic changes in the rates of amino acid transport by marine invertebrate larvae (Echinodermata, Echiura, Mollusca). *Biol. Bull.* **176:** 161–168.

Manahan, D. T. 1990. Adaptations by invertebrate larvae for nutrient acquisition from seawater. *Am. Zool.* **30:** 147–160.

Mann, R., and S. M. Gallager. 1985. Physiological and biochemical energetics of larvae of *Teredo navalis* L. and *Bankia gouldi* (Bartsch) (Bivalvia: Teredinidae). *J. Exp. Mar. Biol. Ecol.* **85:** 211–228.

McClintock, J. B., and J. S. Pearse. 1986. Organic and energetic content of eggs and juveniles of antarctic echinoids and asteroids

with lecithotrophic development. *Comp. Biochem. Physiol.* **85A**: 341–345.

McEdward, L. R., and F.-S. Chia. 1991. Size and energy content of eggs from echinoderms with pelagic lecithotrophic development. *J. Exp. Mar. Biol. Ecol.* **147**: 95–102.

Olson, R. R., I. Bosch, and J. S. Pearse. 1987. The antarctic larval food limitation hypothesis examined for the asteroid *Odontaster validus. Limnol. Oceanogr.* **32**: 686–690.

Parsons, T. R., Y. Maita, and C. A. Lailli. 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*, Pergamon Press, Oxford.

Pearse, J. S. 1969. Slow developing demersal embryos and larvae of the antarctic sea star *Odontaster validus. Mar. Biol.* **3**: 110–116.

Pearse, J. S., J. B. McClintock, and I. Bosch. 1991. Reproduction of antarctic benthic marine invertebrates. *Am. Zool.* **31**: 65–80.

Rivkin, R. R. 1991. Seasonal patterns of planktonic production in McMurdo Sound, Antarctica. *Am. Zool.* **31**: 5–16.

Rivkin, R. R., I. Bosch, J. S. Pearse, and E. J. Lessard. 1986. Bacterivory: a novel feeding mode for asteroid larvae. *Science* **223**: 1311–1314.

Schmidt-Nielsen, K. 1984. *Scaling: Why Is Animal Size So Important?* Cambridge University Press, Cambridge.

Shilling, F. M., and D. T. Manahan. 1990. Energetics of early development for the sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus* and the crustacean *Artemia* sp. *Mar. Biol.* **106**: 119–127.

Shilling, F. M., and I. Bosch. 1994. 'Pre-feeding' embryos of antarctic and temperate echinoderms use dissolved organic material for growth and metabolic needs. *Mar. Ecol. Prog. Ser.* **109**: 173–181.

Stephens, G. C. 1988. Epidermal amino acid transport by marine invertebrates. *Biochim. Biophys. Acta.* **947**: 113–138.

Strathmann, M. F. 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast: Data and Methods for the Study of Eggs, Embryos, and Larvae.* University of Washington Press, Seattle and London. 670 pp.

Torres, J. J., and G. N. Somero. 1988. Metabolism, enzymic activities and cold adaptation in Antarctic mesopelagic fishes. *Mar. Biol.* **98**: 169–180.

Welborn, J. R., and D. T. Manahan. 1991. Seasonal changes in concentrations of amino acids and sugars in seawaters of McMurdo Sound, Antarctica: uptake of amino acids by asteroid larvae. *Antarctic J. U.S* **26**: 160–162.

Wright, S. H. 1988. Nutrient transport across the integument of marine invertebrates. Pp. 173–218 in *Advances in Comparative and Environmental Physiology*, Vol. 2, R. Gilles, ed. Springer-Verlag, Berlin.

Wright, S. H., and D. T. Manahan. 1989. Integumental nutrient uptake by aquatic organisms. *Ann. Rev. Physiol.* **51**: 585–600.